Intraspecific and interspecific growth variation of ectomycorrhizal fungi at different temperatures

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Received November 12, 1986

CLINE, M. L., FRANCE, R. C., and REID, C. P. P. 1987. Intraspecific and interspecific growth variation of ectomycorrhizal fungi at different temperatures. Can. J. Bot. **65**: 869–875.

Geographically distinct isolates of the ectomycorrhizal fungi *Pisolithus tinctorius, Cenococcum geophilum, Thelephora terrestris,* and *Suillus granulatus* showed definite temperature optima for growth in pure culture. Temperatures promoting the greatest fungal growth varied interspecifically and intraspecifically over the temperature treatments of 16, 21, 27, 32, and 38°C. Of the four fungal species, *Pisolithus tinctorius* exhibited the greatest growth at the higher temperature treatments, with growth optima for all isolates occurring between 21 and 32°C. The maximum temperature for growth of *Cenococcum geophilum* was 27°C, with optimal mycelial growth occurring between 16 and 27°C, depending upon the particular isolate considered. *Suillus granulatus* showed greatest mycelial growth at 27°C and below, with temperature optima for the different isolates ranging from 16 to 32°C. The pure-culture growth of *Thelephora terrestris* was high relative to the other fungal species examined with growth optima between 21 and 27°C. The degree of intraspecific variation of mycelial growth in response to temperature was high for all fungal species, indicating the existence of physiologically distinct genotypes. Attempts to relate fungal growth performance to geographic origin of isolate showed a trend for *Pisolithus tinctorius*. However, no such relationships were apparent for the other species.

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Des isolats de champignons ectomycorhiziens d'origines géographiques distinctes appartenant aux espèces *Pisolithus tinctorius, Cenococcum geophilum, Thelephora terrestris* et *Suillus granulatus* ont montré des optimums de température bien définis pour la croissance en culture pure. Les températures qui donnent un maximum de croissance fongique varient interspécifiquement et intraspécifiquement avec des températures de 16, 21, 27, 32 et 38°C. Parmi les quatre espèces fongiques, le *P. tinctorius* montre la plus forte croissance aux hautes températures avec des optimums de croissance pour toutes les souches situés entre 21 et 32°C. La température maximum pour la croissance du *C. geophilum* est de 27°C avec une croissance optimale du mycélium se situant entre 16 et 27°C, selon la souche particulière considérée. Le *S. granulatus* montre la plus forte croissance mycélienne à 27°C et moins, avec des optimums de température pour différentes souches entre 16 et 32°C. La croissance en culture pure du *T. terrestris* est élevée comparativement aux autres espèces fongiques examinées et les optimums se situent entre 21 et 27°C. Le degré de variation intraspécifique dans la croissance du mycélium, en réaction à la température, est élevé pour toutes les espèces fongiques, ce qui indique l'existence de génotypes physiologiquement distincts. Les tentatives effectuées pour essayer de relier la performance de croissance des champignons avec l'origine géographique des souches montrent une tendance pour le *P. tinctorius*. Il n'existe pas de telles relations chez les autres espèces.

[Traduit par la revue]

Introduction

Temperature and genotype exert a profound influence on ectomycorrhizal (ECM) fungal growth, metabolism, and root colonization (4, 11, 12, 21, 22). The manner in which these two factors interact strongly influences the growth limits of fungal species and strains of species.

Temperature optima for colonization and infestation of roots by ECM fungi may differ from optimal temperatures for fungal growth in pure culture (20). Different nutrient media can influence temperature optima for ECM fungal growth in pure culture (4). Similar findings led Theodorou and Bowen (21) to conclude that fungal growth in pure culture may be of limited value for predicting fungal behavior in the rhizosphere.

Conversely, Marx and Davey (10) reported that temperatures most favorable for ECM synthesis by the fungal symbionts *Leucopaxillus cerealis* var. *piceina* (Peck) ined. and *Pisolithus tinctorius* (Pers.) Coker & Couch on *Pinus echinata* Mill. seedlings in aseptic culture closely corresponded to the temperatures promoting the greatest fungal growth in pure culture. Similar work with the ECM fungus *Thelephora terrestris* (Ehrh.) Fr. showed that the temperature for optimal growth in pure culture was the same temperature yielding the greatest ectomycorrhizae formation of *Pinus taeda* L. seedlings (11). In contrast, *Pisolithus tinctorius* grew best in pure culture at 28°C but produced the highest percentage of ectomycorrhizae on *Pinus taeda* at 34°C (11). In addition, Marx and co-workers demonstrated that pine seedlings possess a differential ability to form ectomycorrhizae at different temperatures between different isolates of *T. terrestris* and *Pisolithus tinctorius*. The isolate of each fungus forming the greatest mycorrhizae percentage also exhibited the greatest growth in pure culture. Whether pure-culture growth can be used to predict the ectomycorrhizae-forming ability of a particular fungi is a point of contention.

Temperature requirements of ECM fungi appear to vary as much or more between ecotypes of given species as between

Printed in Canada / Imprimé au Canada

TABLE 1. Isolate origin for ectomycorrhizal fungi used in the temperature–isolate experiment. Ordering of isolates within a species corresponds to a north-to-south latitudinal reference according to origin

Isolate	Location
Pisolithus tinctorius	
Pt 158	Griffin State Nursery, WI
Pt 145	Coal spoil; Dickerson County, KY
Pt 098	Coal spoil; Berea, KY
Pt 177	Charlottesville, VA
Pt 166	North Carolina
Pt 185	Ft. Towson Nursery, OK
Pt 133	Athens, GA
Pt 136	Athens, GA
Pt 180	Athens, GA
Pt 138	Athens, GA
Pt 001	Athens, GA
Cenococcum geophilum	
Cg 20	Helsinki, Finland
Cg 13	Helsinki, Finland
Cg 4	Helsinki, Finland
Cg 10	Helsinki, Finland
Cg 21	Helsinki, Finland
Cg 5	Moselle, France
Cg 1	Innsbruck, Austria
Cg 51	Prealps, Switzerland
Cg 2	France
Cg 149	Tieton River, WA
Cg 167	Packwood, WA
Cg 148	Status Pass, WA
Cg 150	Tombstone Pass, OR
Cg 166	Mary's Peak, OR
Cg 161	Tombstone Pass, OR
Cg 145	Blue River, OR
Cg 144	Willow Creek, OR
Cg 347	Athens, GA
Cg 146	Athens, GA
Cg 3	Western Himalayas, India
Suillus granulatus	
Sg 37	Stockholm, Sweden
Sg 20	Ellensburg, Washington
Sg 3	Innsbruck, Austria
Sg 55	Valais, Switzerland
Sg 5	Turin, Italy
Sg 1	Genessee Mountain, CO
Sg 2	Mount Evans, CO
Sg 876	Sandia Mountains, NM
Thelephora terrestris	
Tt 201	Athens, GA
Tt 142	Canby Nursery, OR
Tt 227	Linköping, Sweden

species (5, 16, 17, 22). Mountain isolates of the fungal species *Suillus variegatus* (Batsch ex Fr.) Fr. exhibited lower temperature optima for growth than isolates secured from low elevations (17). Moser (16) also found high elevational isolates of *S. plorans* (Roll.) Sing. better adapted to cold temperatures than lowland isolates of the fungus. Theodorou and Bowen (21) observed large differences in length of root colonization and intensity of growth among strains within several fungal species on *Pinus radiata* D. Don root systems in response to temperatures. Graham and Linderman (3) observed that the slower the growth rate of *Cenoccocum geophilum* Fr. *in vitro*, the fewer the ectomycorrhizae formed on young seedlings inoculated with the isolate.

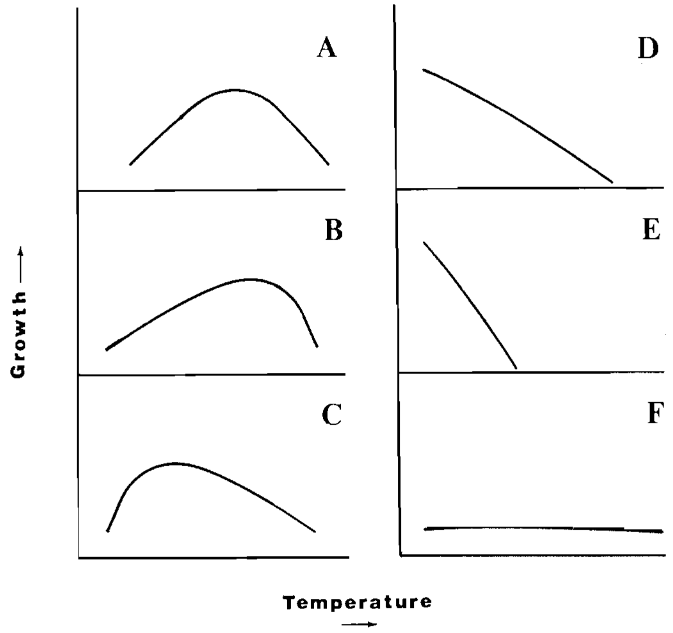

FIG. 1. Generalized models of pure-culture growth patterns exhibited by ectomycorrhizal fungi in response to increasing culture temperature over the range 16–38°C.

According to Trappe (22), temperature requirements of various isolates must be determined to (*i*) grow inoculum effectively; (*ii*) attain good mycorrhizae development in the nursery after inoculation; and (*iii*) obtain good survival and growth after outplanting. Comprehensive comparisons of different isolates of fungal species will certainly reveal ecotypes better suited for nursery inoculation than others. The present study examines pure-culture growth of a number of geographically distinct isolates of four ECM fungal species in response to temperature. This paper focuses on genetic considerations and temperature and the interaction of these two factors.

Methods

Isolates of *Pisolithus tinctorius*, *C. geophilum*, *T. terrestris*, and *S. granulatus* (L. ex Fries) O. Kuntze were screened for growth at various temperatures. Isolates were collected from a range of tree hosts growing on geographically diverse international sites and had been in culture for various time periods (Table 1).

The effect of temperature on pure-culture growth of ECM fungi was assessed using liquid-culture Petri plate systems. Systems consisted of glass beads (5 mm diameter) bathed in modified Melin–Norkrans (MMN) nutrient solution (8). A nylon mesh disc placed over the glass beads was used as the growing surface for the fungi. Solution was maintained at a depth which allowed wetting of the mesh but avoided submergence.

Liquid media, glass beads, and nylon mesh were steam sterilized prior to construction of the systems within a sterile transfer hood. One 7-mm disc of fresh mycelium of each isolate, removed from pure cultures of fungi actively growing on MMN agar, was centrally placed on the nylon mesh in each system. Plates were then wrapped in Mylar to minimize water loss by evaporation and thereby maintain a more constant water potential. Three replicate Petri plate systems of each fungal isolate were utilized at each temperature examined.

Plates were placed in incubators without light. Mycelial growth was examined at temperatures of 16, 21, 27, 32, and 38°C (±1°C), based on previous studies of ECM fungi on laboratory media which indicated that optimal growth temperature is between 18 and 29°C (4, 21). Mycelial mat extension along two predetermined perpendicular axes was used as a measure of fungal growth response.

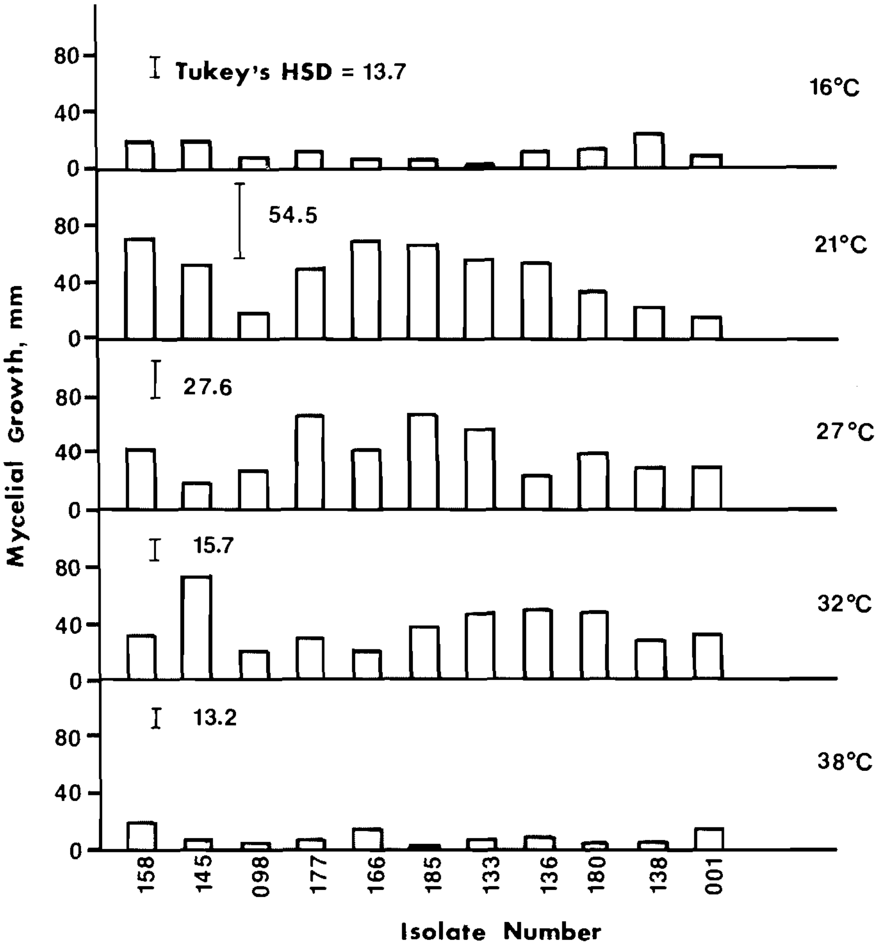

FIG. 2. Pure-culture growth at 6 weeks for *Pisolithus tinctorius* isolates at 16, 21, 27, 32, and 38°C. Isolates are arranged north (left) to south (right) with respect to geographic origin.

Cumulative extension was measured three times per week. Growth was allowed to continue until (*i*) mycelium expanded to the limits of the Petri plate; (*ii*) the treatment was terminated if the fungus showed no growth after 6 weeks in any replicate within a temperature treatment for 10 consecutive days; or (*iii*) the treatment was terminated at 70 days for all cultures remaining in active growth but not extending to the limits of the Petri plate.

Mycelial growth data were used to calculate total cumulative growth at 6 weeks for each fungal isolate in each temperature treatment. Fungal growth recorded after 6 weeks was negligible as indicated by staling of cultures. Statistical analysis involved two-way analysis of variance (ANOVA) with the main effects of temperature and fungal isolate for each fungal species. Multiple comparison of treatment means was accomplished with Tukey's test for honestly significant differences (HSD). All testing was performed at $\alpha = 0.05$.

Results

Nearly all isolates of each fungal species exhibited mycelial growth that followed one of six general patterns of growth in relation to temperature (Fig. 1). Figure 1A shows a normal distribution of growth over the range of treatment temperatures, while Figs. 1B and 1C are skewed to the left (i.e., higher temperature optimum) and right (i.e., lower temperature optimum), respectively. The skewed side represents that half of the distribution with least development. Figures 1D and 1E represent growth patterns for which only a portion of the entire growth spectrum was discerned from the treatment temperatures. Figure 1D shows a gradual decrease in growth with increased temperature and Fig. 1E shows a rapid decrease to zero growth with increased temperature. Figure 1F shows a constant low level of growth over all temperatures.

Pisolithus tinctorius

Figure 2 shows the mean mycelial growth for the temperature × isolate interaction involving 5 temperatures and 11 isolates of *P. tinctorius*. Although growth was evident for all isolates within each temperature treatment, greater growth occurred at 21, 27, and 32°C. The optimal temperature for growth of *P. tinctorius* varied with the isolate. No *P. tinctorius* isolate exhibited optimal growth at 16°C. However, isolates Pt 158, Pt 145, and Pt 138 showed the greatest relative growth. Isolates which had greater growth at 21°C were Pt 158, Pt 166, and Pt 185. Isolates Pt 177, Pt 185, and Pt 133 possessed the greatest growth at 27°C. Isolate Pt 145 performed best at 32°C. Although all isolates had reduced mycelial growth at 38°C, Pt 098, Pt 185, Pt 180, and Pt 138 exhibited very restricted growth.

Three general patterns of growth from 16 to 38°C were exhibited with the 11 geographically distinct isolates of *P. tinctorius*. The normal distribution growth pattern (Fig.1A) was characteristic of Pt 098, Pt 177, and Pt 133 (all with optima at 27°C). Negative skewness (Fig. 1B) was evident with Pt 138 (optimum 27°C), Pt 180 (optimum 32°C), and Pt 001 (optimum 32°C, less kurtosis). Positive skewness (Fig. 1C) occurred in the growth patterns of Pt 158 (optimum

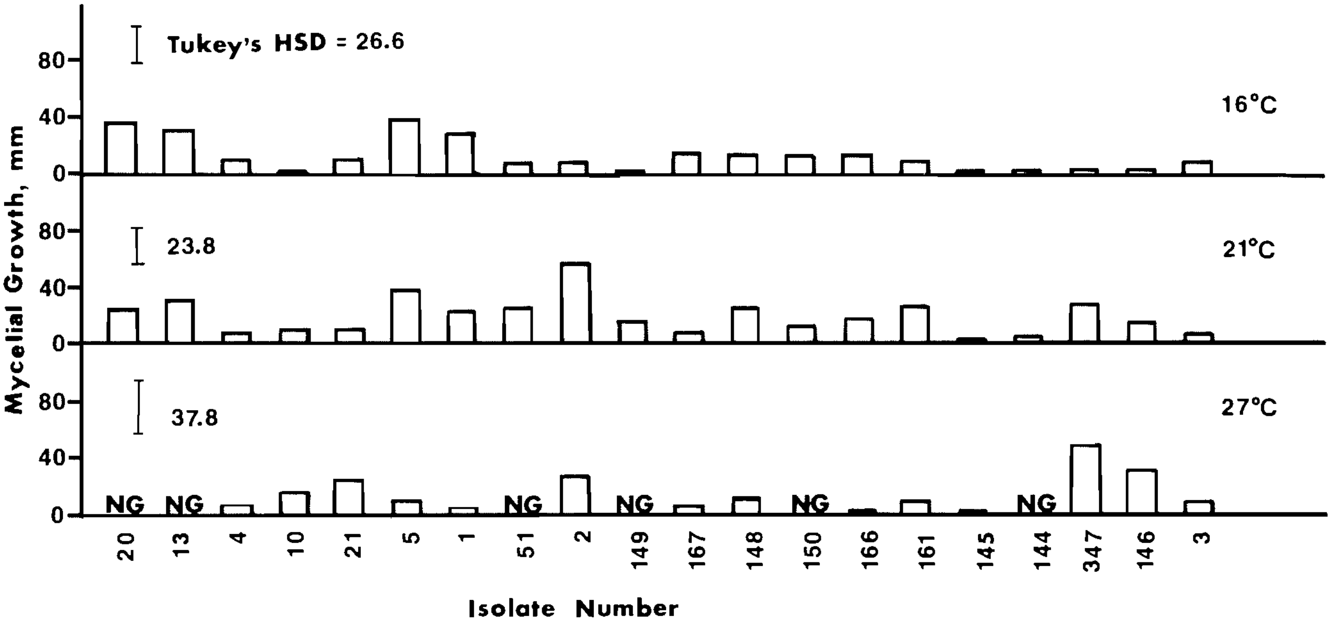

FIG. 3. Pure-culture growth at 6 weeks for *Cenococcum geophilum* isolates at 16, 21, 27, 32, and 38°C. Isolates are arranged north (left) to south (right) with respect to geographic origin. NG, no growth. Isolate Cg 146 was the only isolate to grow at 32°C (< 10 mm). No isolate grew at 38°C.

21°C), Pt 166 (optimum 21°C), and Pt 185 (optimum 21 and 27°C). Isolates Pt 145 (optimum 32°C) and Pt 136 (optimum 21°C) showed variable growth with temperature.

Differences in the inherent growth rate of different isolates of *P. tinctorius* are evident when optimal growth rates of each isolate are compared (Fig. 2). Aside from growth optima at different temperatures, *P. tinctorius* isolates exhibited quantitative growth differences at each of their optima. Comparison of mycelial growth over the range of temperature treatments and optimal growth temperatures for each isolate (Fig. 2) with the geographic origin of each isolate (Table 1) suggests a growth–origin relationship based on temperature and latitudinal distribution. Isolates from northern origins (left in Fig. 2) generally possessed growth optima at lower temperatures than isolates from southern origins (right in Fig. 2).

Cenococcum geophilum

Mycelial growth of *C. geophilum* occurred primarily at 16, 21, and 27°C (Fig. 3). No growth occurred at 38°C, and only 1 of 20 isolates grew at 32°C (Cg 146, less than 10 mm diameter mycelial growth). As with *P. tinctorius*, the temperature treatment promoting optimal growth of *C. geophilum* varied with fungal isolate. Isolates Cg 20, Cg 13, Cg 5, and Cg 1 grew well at 16°C, although some of these isolates showed equally good growth at other temperatures. Conversely, several isolates showed poor growth at 16°C (i.e., Cg 10, Cg 149, Cg 145, Cg 144, Cg 146, and Cg 347). Mycelial growth of isolates Cg 5 and Cg 2 exceeded all other isolates at 21°C. Isolates Cg 21, Cg 2, Cg 347, and Cg 146 exhibited the greatest growth at 27°C. Several isolates were unable to grow at 27°C (i.e., Cg 20, Cg 13, Cg 51, Cg 149, Cg 150, and Cg 144).

The temperature treatments selected appeared to be at the upper extreme for growth of *C. geophilum*, since essentially no growth occurred above 27°C and most isolates grew at 16°C. The 20 fungal isolates examined exhibited four patterns of growth over the temperature range of 16 to 27°C. Patterns of mycelial growth for *C. geophilum* included (*i*) normal distribution (Fig. 1A) for the French isolate Cg 2 (optimum at 21°C) and the Pacific Northwest isolates Cg 148 and Cg 161 (both with optimum 21°C); (*ii*) negative skewness (Fig. 1B) for the Finnish isolates Cg 10 and Cg 21 (both with optimum 27°C), the Austrian isolate Cg 51 (optimum 21°C), the Washington isolate Cg 149 (optimum 21°C), and Georgia isolates Cg 347 and Cg 146 (both with optimum 27°C); (*iii*) gradual decline in growth with increasing temperature (Fig. 1D) for French isolate Cg 5 (optimum 16 and 21°C), the Austrian isolate Cg 1 (optimum 16°C), and Pacific Northwest isolates Cg 145 and Cg 166 (both with optimum 21°C) and Cg 167 and Cg 144 (both with optimum 16°C); (*iv*) rapid or sudden decline in growth with increasing temperature (Fig. 1E) for the Finnish isolates Cg 20 and Cg 4 (both with optimum 16°C), Cg 13 (optimum between 16 and 21°C), and the Pacific Northwest isolate Cg 150 (optimum 16 and 21°C); and (*v*) constant low level of growth over all temperatures (Fig. 1F) for the Indian isolate Cg 3 and the Pacific Northwest isolate Cg 145 (both with no distinctive optimum). Isolates exhibiting optimal growth at 16°C in this study may actually have growth optima at lower temperatures, but these were not examined in the present study.

Suillus granulatus

Suillus granulatus mycelial growth occurred primarily at 16, 21, 27, and to a limited extent 32°C (Fig. 4). No growth was exhibited by any isolate at 38°C. Optimal temperature for fungal growth varied with isolate. No significant difference in growth occurred between isolates at 16°C. At 21°C, isolates Sg 37 and Sg 5 exhibited the greatest growth. Isolates Sg 1 and Sg 37 produced the most growth at 27°C. Mycelial growth at 32°C occurred only with isolates Sg 2, Sg 3, and Sg 37.

Four general growth patterns, relative to temperature, were evident for the eight isolates of *S. granulatus*. Isolates Sg 5 and Sg 20 (both with optimum 21°C) and Sg 3 (optimum 27°C) exhibited a normal distribution (Fig. 1A). Negative skewness (Fig. 1B) occurred with isolates Sg 2 (optimum 32°C) and Sg 1 and Sg 55 (both with optimum 27°C). Isolate 37 (optimum

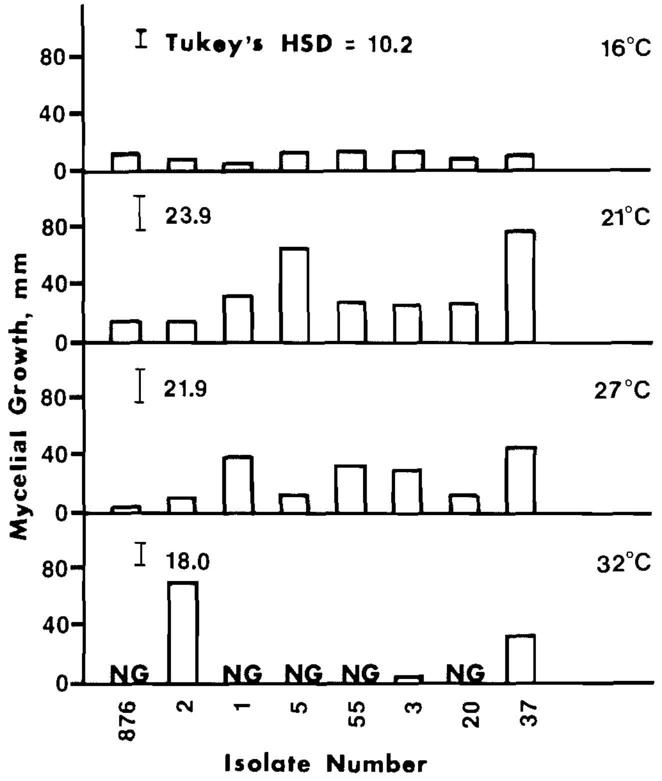

FIG. 4. Pure-culture growth at 6 weeks for *Suillus granulatus* isolates at 16, 21, 27, 32, and 38°C. Isolates are arranged north (left) to south (right) with respect to geographic origin. NG, no growth. No isolate grew at 38°C.

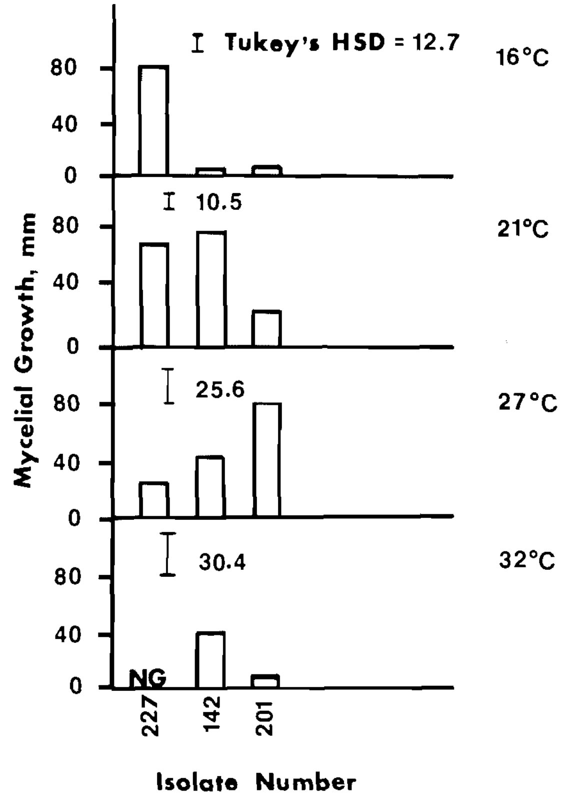

FIG. 5. Pure-culture growth at 6 weeks for *Thelephora terrestris* isolates at 16, 21, 27, 32, and 38°C. Isolates are arranged north (left) to south (right) with respect to geographic origin. NG, no growth.

21°C) showed a positive skewness (Fig. 1C). A gradual decline in growth with increasing temperature (Fig. 1D) was characteristic of Sg 876 (optimum 16°C).

Thelephora terrestris

Two isolates of *T. terrestris* (Tt 227 and Tt 201) exhibited mycelial growth at all treatment temperatures except 38°C (Fig. 5). Isolate Tt 142 showed some growth at 38°C. Isolate Tt 227 showed the greatest growth at 16°C, while isolates Tt 227 and Tt 142 both grew well at 21°C. At 27°C isolate Tt 201 showed the greatest growth. Isolate Tt 142 was the best performing *T. terrestris* isolate at 32°C, while Tt 227 did not grow at this temperature.

Each isolate exhibited a different growth pattern over the temperature range investigated. Isolate Tt 201 displayed a negatively skewed growth pattern (Fig. 1A). A gradual decline in growth with increased temperatures was apparent for Tt 227 (optimum 21°C) (Fig. 1D). Tt 142 (optimum 21°C) exhibited positive skewness (Fig. 1B).

The total growth of each fungal species (all isolates combined) was greatly different after 42 days. *Thelephora terrestris* produced the most growth. *Cenococcum geophilum* was very slow growing in pure culture relative to the other species examined. Mycelial growth of *S. granulatus* and *P. tinctorius* was intermediate in cumulative growth.

Discussion

The high degree of variability in pure culture mycelial growth between isolates of the same fungal species in response to temperature suggests that fungal genotype can significantly influence growth study results. Characterization of fungal growth based on the performance of one or two isolates can be misleading. Trappe (22) states that until more isolates of each fungal species can be examined, overall differences between species cannot be assessed beyond what can be inferred from their natural distribution. The range of temperatures at which fungal growth occurred in the present study aids in definition of thermal limits for pure-culture growth of each of the four ECM fungal species. For many of the isolates of *C. geophilum*, *S. granulatus*, and *T. terrestris*, the entire profile of temperatures promoting mycelial growth was not sampled, and therefore, only the maximum temperature limits can be considered. Although the absolute limits for mycelial growth of *P. tinctorius*, both high and low, were not attained in the study, the gradient of temperatures examined appears to include temperature optima for all isolates. No attempt was made to determine temperatures lethal to each fungal species; however, previous results have shown that these ECM fungi are capable of growth after exposure to subfreezing temperatures (1).

Pure-culture growth performance showed *P. tinctorius* to be more tolerant of high temperatures than the other fungi examined, which is in agreement with previous results showing *P. tinctorius* abundant in soils with high surface temperatures (9, 19). However, certain changes in growth habit were noted for *P. tinctorius* over the range of temperatures investigated. Vegetative mycelium changed in color from golden brown at low temperatures (16–27°C) to bright yellow at high temperatures (32 and 38°C), with the liquid culture media rapidly darkening to burnt orange. In addition, at higher temperatures growth "staled" sooner than at lower temperatures. The pH of "staled" cultures had dropped from an initial value of 5.5 to 2.7–3.5 and may have been responsible for prohibiting further growth. Giltrap and Lewis (2) observed more dry weight growth of *C. geophilum* in pure culture when the nutrient solution was buffered to maintain a pH above 5 versus unbuffered solution which dropped to a pH value of 2.7.

The temperature promoting optimal pure-culture growth of *P. tinctorius* occurs between 21 and 32°C, depending on which isolate is considered. Other authors have reported a pure-culture optimal growth temperature for *P. tinctorius* of 28°C (4, 11). Results of the present study show that the optimal temperature for pure-culture growth can vary substantially depending on which isolate is being examined.

The fungal isolate is responsible for large differences in inherent growth and optimal growth temperature. Results suggest that optimal temperatures for growth of *P. tinctorius* isolates in pure culture reflect environmental conditions at the site of origin. All *P. tinctorius* isolates originated from the continental United States; however, more northerly isolates generally had lower temperature optima for mycelial growth, while the more southerly isolates had higher temperature optima.

Although variation in pure-culture growth between isolates of *P. tinctorius* was large, differences in growth among the fungal species were much greater. Even so, the intraspecific genetic variation would be sufficiently large to misconstrue comparisons of ecological adaptability of entire fungal species, based on only a few isolates. Different isolates of *P. tinctorius* were responsible for differences in cumulative growth, growth temperature optima, and growth pattern over the range of temperatures.

Results for pure-culture mycelial growth indicate that *C. geophilum* is intolerant of temperatures of 32°C and above. In contrast, Wright (23) contends that growth of *C. geophilum* is favored by relatively high temperatures, which he feels helps to explain its absence in shaded areas. However, in the southeastern United States, *C. geophilum* is not excluded from shaded areas occurring on sites supporting loblolly pine. Trappe (22) found *C. geophilum* to be broadly adapted to temperature extremes. Mikola (15) reported poor mycelial growth of *C. geophilum* in pure culture at 10–15°C. However, he found it commonplace in spruce forests of northern Finland, where the ground remains frozen until midsummer and soil temperatures rarely reach 20°C.

The distinct demarcations in the natural distribution of *C. geophilum* may stem from its inherent slow growth and inability to compete with other fungi under certain forest conditions. The tolerance of *C. geophilum* to water stress (13, 14, 18, 23) may ecologically compensate for its slow growth rate and allow *C. geophilum* to compete effectively in more xeric environments where many other soil microflora cannot survive. Depending on which isolate was considered, *C. geophilum* exhibited greatest mycelial growth at 16, 21, and 27°C. Theodorou and Bowen (21) reported optimal pure culture linear growth at 20 and 25°C with a significant decline at 16°C.

Fungal growth of all isolates examined appeared to be distributed normally with respect to temperature or skewed to the positive or negative. Those isolates which did not fit this description possessed spectra of growth temperatures not totally encompassed in the present study or showed random variation. Negative skewness would indicate a sharp decline in growth at the higher temperature limit (i.e, growth optimum at a lower temperature), while positive skewness would indicate a sharp decline in the low temperature extreme (i.e., growth optimum at a higher temperature). Such sharp declines may result from inhibitory effects of temperature extremes on metabolism and (or) changes in membrane integrity (6, 7). Often sharp breaks in the growth of living organisms are associated with temperature as the result of membrane phase separations (7). Regardless of the mechanism responsible, these differences illustrate genetic variation in fungal growth and tolerance to temperature at the interspecific and intraspecific levels.

Genetic variability in mycelial growth among *C. geophilum* isolates was great; however, no distinct relationship was discerned relating mycelial growth at different temperatures with geographic latitudinal origin. This does not mean that such a relationship does not exist. The isolates of *C. geophilum* were obtained from international sources. The variation in the elevational spectrum encountered over the latitudinal continuum may have masked a north–south relationship.

Suillus granulatus, which is generally indigenous to less extreme sites than *C. geophilum* and *P. tinctorius*, exhibited most mycelial growth at temperature treatments of 27°C and below; however, growth at 32°C was recorded for three isolates. Total mycelial growth and the temperature range over which growth occurred appeared intermediate with respect to other fungi examined. Theodorou and Bowen (21) examined three isolates of *S. granulatus* and found that two isolates showed linear growth temperature optima of 25°C and one of 20°C. Growth temperature optima for the eight isolates of *S. granulatus* assessed in the present study ranged from 16 to 32°C.

Several *S. granulatus* isolates exhibited changes in growth habit with temperature. Most mycelial growth was filamentous over the temperature range examined, but some isolates displayed a smooth, dense, glabrous, "callus-like" growth habit at 27 and 32°C. As is the case with *P. tinctorius, S. granulatus* altered the color of the liquid nutrient media to yellow and eventually darkened it to brown. This color intensified at higher growth temperatures.

Pure-culture growth of *T. terrestris* was high relative to the other fungi examined and varied in response to temperature. Although only three isolates were examined, growth temperature optima appeared related to temperatures of the location from which the isolate originated. Isolate Tt 227 exhibited the lowest growth temperature optimum and Tt 201 the highest with Tt 142 intermediate.

Temperature tolerance limits also varied by fungal isolate. Marx *et al.* (11) examined two isolates of *T. terrestris* that displayed linear and dry weight growth optima of 26°C, with a maximum temperature limit of 34°C for one isolate and 30°C for the other. Although a small number of isolates have been examined, variability in the growth of *T. terrestris* appears high from one isolate to another.

Trappe (22) indicates that fungal specialization and diversity will allow selection of specific traits that improve seedling performance in the nursery and field. However, ECM fungal species must be typified based on screening of an appropriate number of isolates to ascertain cardinal environmental limits on performance before selections are made. Results of the present study indicate that particular isolates and (or) species may be better suited than others for at least certain aspects of nursery mycorrhizal inoculation programs (e.g., inoculum production). The great variation in pure-culture growth optima of fungal species and isolates demonstrated in the present study may reflect even greater variation in the ability of specific fungal species or isolates to colonize root systems.

Acknowledgments

We thank the following people for their assistance in obtaining fungal isolates: B. K. Bakshi, B. Fassi, L. Froideveux, L. S. Gilman, M. B. Hosford, F. LeTacon, G. Lundeberg, D. H. Marx, P. Mikola, R. Molina, M. M. Moser, J. G. Palmer, J. W. Riffle, and J. M. Trappe.

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